The Post-Docking Role of Synaptotagmin 1 and Munc 18-1 In Mouse Chromaffin Cells

Kedar, G.H.

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English Summary

Brain is the chauffer of all five senses that governs our daily experience, right from viewing beautiful Tulip garden to tasting mouth-watering cheese. It is also responsible for cognition and controlling involuntary activities such as heart functioning and digestion. Neuronal cells (and other cells for that matter) ceaselessly communicate with each other to maintain the vital functions of body. One of the fundamental mode of cellular communication is through releasing synaptic/secretory vesicles. These synaptic/secretory vesicles mainly contain neurotransmitters, hormones, amines and peptides, that act like messengers important for impeccable communication. Apart from mode and matter of communication, temporal release is also essential. For explicit temporal release of cellular communication, different proteins play central role in vesicle fusion process. Syt1, Munc18-1, SNAP25 and Syntaxin1 are found to be important for docking and fusion. In addition, apart from SNAP25 and Syntaxin1, Synaptobrevin2 is a significant part of SNARE complex that is evolutionally critical from yeasts to humans for membrane fusion.

We focused on exploring Syt1s role in secretory vesicle docking in mouse chromaffin cells. Syt1 play important role in secretory vesicle docking by binding to minimal acceptor complex (SNAP25:Syntaxi1) (de Wit et al., 2009). We attempted to understand specific Syt1-C2B bottom domains role in docking. Mutation in this particular region (R398, R399) previously found to disrupt neurotransmitter release in *syt1 null* hippocampal neurons (Xue, Ma, Craig, Rosenmund, & Rizo, 2008). We found that, Syt1-C2B bottom face is dispensable for secretory vesicle docking and in living cells, probably does not need PIP2 to dock vesicles (Kedar et al., 2015). We further used different mutants of multiple regions like Ca^{2+} binding loop, polybasic region and also individual C2 domains to identify regions directly involved in secretory vesicle docking. Total number, vesicle distribution and docking were unchanged compared to control. We conclude that, all the studied mutant versions of Syt1 in this chapter are dispensable for secretory vesicle docking.

We also studied another protein important for secretory vesicle docking and fusion i.e. Munc18-1. Previously, it was found that Munc18-1s domain 3a is crucial for Synaptobrevin2 binding and define inhibitory or stimulatory vesicle fusion activity. With anticipation of having effect on secretory vesicle docking, we used domain 3a mutants in our docking assay. We found that these mutants are dispensable for docking as total number, vesicle distribution and docking was normal. Electrophysiology and liposome assay confirms that Munc18-1s domain 3a 12th α-helix leads to opening of closed confirmation of Syntaxin-1, followed by productive SNARE-complex assembly by allowing Synaptobrevin2 to interact and vesicle priming.

Apart from identifying specific proteins role in secretory vesicle docking and fusion,

we also strived to bring new method to screen or analyze secretory vesicle docking with the help of high-resolution optical microscopy. We utilized 3D-SIM microscopy to generate optical images and validated docking analysis by two different plugins, which work on two completely independent platforms. We found that, difference in vesicle and plasma membrane marker matched EM generated docking data for *wild type* and *syt1 null* chromaffin cells. This method is promising for screening or pilot analysis of secretory vesicle docking and select experimental conditions of interest for further docking analysis using high quality but low through-put TEM experiments.